The expression of terminal deoxynucleotidyl transferase and paired box gene 5 in Merkel cell carcinomas and its relation to the presence of Merkel cell polyomavirus DNA

Benjamin Sundqvist
Faculty of Medicine

Helsinki 15.10.2019
Thesis
benjamin.sundqvist@helsinki.fi
Instructor: Tom Böhling
UNIVERSITY OF HELSINKI
Faculty of Medicine

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Tiedekunta – Fakultet – Faculty	Koulutusohjelma – Utbildningsprogram – Degree Programme
Medicinska fakulteten	Utbildningsprogrammet i medicin

Tekijä – Författare – Author
Benjamin Sundqvist

Työn nimi – Arbetets titel – Title
The expression of terminal deoxynucleotidyl transferase and paired box gene 5 in Merkel cell carcinomas and its relation to the presence of Merkel cell polyomavirus DNA

Oppiaine/Opintosuunta – Läroämne/Studieinriktning – Subject/Study track

Työn laji – Arbetets art – Level	Aika – Datum – Month and year	Sivumäärä – Sidoantal – Number of pages
Fördjupade studier	15.10.2019	7

Tiivistelmä – Referat – Abstract

Merkelcellskarcinom (MCC) är en ovanlig och agressiv neuroendokrin hudcancer vars cellulära ursprung är okänt. Genomet av Merkel cell polyomavirus (MCPyV) är integrerat i genomet av tumörcellerna i cirka 80% av MCC-tumörer. Baserat på proteinexpressionsmönster anses cellerna i MCC vara närmast besläktade med Merkelceller (mekanoreceptiva celler i huden). Av denna orsak har Merkelceller antagits vara ursprungscellen för MCC men detta har aldrig bevisats. Höga grader av PAX5- och TdT-expression har observerats i MCC, detta tyder på möjligheten att ursprungscellen för MCC inte är den postmitotiska Merkelcellen utan snarare en pro/pre- eller pre-B cell eftersom co-expression av PAX5 och TdT är begränsat till dessa celler under fysiologiska omständigheter. I denna studie undersöktes expressionen av PAX5 och TdT i 117 MCC-tumörer med immunohistikemi. Utav de 117 tumörerna som undersöktes expresserade 31.6% TdT, 22.2% expresserade PAX5 och graden av co-expression var 11.1%. Dessa expressionsgrader är betydligt lägre än vad som observerats i tidigare studier. Vidare observerades ett statistiskt signifikant samband mellan expressionen av TdT och närvaro av MCPyV DNA samt mellan expressionen av TdT och antalet kopior av MCPyV-genomet i tumörvävnaden. Inget statistiskt signifikant samband observerades mellan expressionen av PAX5 och/eller TdT och prognos vilket underminerar användningen av dessa markörer i kliniskt syfte.

Avainsanat – Nyckelord – Keywords
Merkel cell carcinoma; Merkel cell polyomavirus; Terminal deoxynucleotidyl transferase; Paired box gene 5

Ohjaaja tai ohjaajat – Handledare – Supervisor or supervisors
Prof. Tom Böhling

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

Received: 20 March 2018 | Revised: 22 September 2018 | Accepted: 8 October 2018

DOI: 10.1111/cup.13372

WILEY JCP JOURNAL OF CUTANEOUS PATHOLOGY

ORIGINAL ARTICLE

The expression of terminal deoxynucleotidyl transferase and paired box gene 5 in Merkel cell carcinomas and its relation to the presence of Merkel cell polyomavirus DNA

Benjamin Johansson[1] | **Helka Sahi**[2] | **Virve Koljonen**[3] | **Tom Böhling**[1]

[1]Department of Pathology, Helsinki University and HUSLAB, Helsinki, Finland

[2]Department of Dermatology and Allergology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[3]Department of Plastic Surgery, Helsinki University Hospital, Helsinki, Finland

Correspondence
Virve Koljonen, Department of Plastic Surgery, Töölö Hospital, P.O. Box 266, FI-00029 HUS, Finland.
Email: virve.koljonen@hus.fi

Background: Merkel cell carcinoma (MCC) tumor samples frequently express B-lymphoid lineage markers. However, the reasons for expression of specific B-lymphoid lineage markers are still unclear. We studied the expression of TdT and PAX5 (two B-cell lymphoid lineage markers) in a large pool of MCC tissue microarray samples.

Methods: Immunoexpression and staining intensities of TdT and Pax-5 were statistically correlated with patient, tumor, Merkel cell polyomavirus (MCV), and disease-specific parameters.

Results: In a cohort of 117 MCC patients and their corresponding tumor samples, TdT was expressed in 37 (31.6%) samples and PAX5 in 26 (22.2%). Simultaneous immunostaining for TdT and PAX5 was observed in 13 (11.1%) samples. A statistically significant relationship was observed between MCV virus copy number and positive TdT expression ($P = 0.0056$). Similarly, a significant relationship was also observed between positive TdT and tumor MCV virus positivity ($P = 0.000495$).

Conclusion: We observed frequent TdT and PAX5 immunoexpression in MCC tumor samples. However, simultaneous immunoexpression of these markers was scarce. TdT expression was statistically significantly associated with MCV positivity. The absence of a statistically significant association between tumor parameters and disease progression markers undermines the systemic use of these markers in clinical practice.

KEYWORDS

Merkel cell carcinoma, Merkel cell polyomavirus-lymphocyte

1 | INTRODUCTION

Merkel cell carcinoma (MCC) is a neuroendocrine carcinoma of the skin. The cellular origins of this rare and highly aggressive skin cancer subtype are thus far unknown. On the basis of expression patterns, MCC tumor cells are considered to be most closely related to Merkel cells (MC), which are mechanoreceptive cells located in the basal layer of the epidermis. At the dermal-epidermal junction, the basal surfaces of the MCs are closely associated with the terminal bulbs of afferent myelinated nerve fibers. Together, the neuron and the Merkel cell are referred to as Merkel's corpuscle, which is a sensitive mechanoreceptor. In the vast majority of the MCCs (approximately 80% of MCC tumors), the DNA genome of Merkel cell polyomavirus (MCV) is integrated in the tumor cell genome; this is considered as the causative agent for tumorigenesis in MCV infection.[1,2] We and others have previously shown significant morphologic and clinicopathological differences between MCV-positive and MCV-negative MCCs.[2–6]

Terminal deoxynucleotidyl transferase (TdT) was one of the first DNA polymerases identified in mammals.[7] DNA polymerases have a vital role in replication, repair, and recombination of nucleic acids. To guide each of these events, the polymerase extends a primer using a DNA or RNA template. However, TdT possesses the unusual ability to incorporate nucleotides without a template. TdT uses only single-stranded DNA as the nucleic acid substrate,[7] thus, synthesizing DNA without a templating strand. Furthermore, TdT has a potential role in the development of certain forms of leukemia. TdT is expressed in immature, pre-B, and pre-T lymphoid cells. TdT is also used as a marker for neoplasms of precursor B-cell and T-cell origin.[8,9]

In addition to lymphoblastic neoplasms, TdT shows nuclear expression in other small round blue cell tumors, such as medulloblastoma, Ewing sarcoma, pediatric rhabdomyosarcoma, and small cell lung carcinoma.[10–12] Very recently, it was shown that TdT protein was expressed in cells of epithelial origin and specifically in sebaceous cells, both benign and malignant.[13] PAX5 is one of nine mammalian Pax transcription factors. Thus far, PAX5 is the only Pax protein expressed within the hematopoietic system.[14] PAX5 is essential for commitment of lymphoid progenitors to the B-lymphocyte lineage[14] and to MCC.[15,16]

Expression of B-lymphoid lineage markers in MCC is frequent (Table 1), including TdT and PAX5. These markers are recurrent in MCC in both primary cutaneous tumors and metastatic lymph nodes.[12,15,16,27–33] Nearly, 90% of the tumors reported express PAX5 and 65% express TdT.[34] On the basis of the frequent expression of B-lymphoid lineage markers, zur Hausen et al.[31] proposed that the cell

TABLE 1 A selection of lymphocyte markers studied in Merkel cell carcinoma from 2005 to 2014

Marker	Normally expressed in	Study	Method	Samples	Positive	MCV+ MCV−
TdT	Pre-B and -T lymphocytes	Bernd et al. (2007)[17]	IHC	28	10	NA
		Sur et al. (2007)[18]	IHC	15	8	NA
		Buresh et al. (2008)[19]	IHC	26	19	NA
		Bhatia et al. (2010)[20]	IHC	23	5	5/17 0/6
		Sidiropoulos et al. (2011)[21]	IHC	40	28	NA
		Murakami et al. (2014)[22]	IHC	30	20	15/20 5/10
		Kolhe et al. (2013)[23]	IHC	27	21	NA
		Zur Hausen et al. (2013)[24]	IHC	16	21	15/18 1/3
PAX5	Pre-B lymphocytes	Dong et al. (2005)[25]	IHC	31	29	20
		Murakami et al. (2014)[22]	IHC	30	30	20/20 10/10
		Kolhe et al. (2013)[23]	IHC	27	24	NA
		Zur Hausen et al. (2013)[24]	IHC	21	21	18/18 3/3
		Mhawech-Fauceglia et al. (2007)[26]	IHC	34	24	NA
Oct2	B- and T-cells, neural cells	Murakami et al. (2014)	IHC	30	27	16/20 9/10
IgG	B lymphocytes	Murakami et al. (2014)	IHC	30	7	7/20 0/10
		Zur Hausen et al. (2013)	IHC	21	10	10/18 0/3
IgA	B lymphocytes	Murakami et al. (2014)[22]	IHC	30	7	7/20 0/10
		Zur Hausen et al. (2013)[24]	IHC	21	10	10/18 0/3
IgM	B lymphocytes	Murakami et al. (2014)[22]	IHC	30	3	3/20 0/10
		Zur Hausen et al. (2013)[24]	IHC	21	6	6/18 0/3
Igkappa	B lymphocytes	Murakami et al. (2014)[22]	ISH (mRNA) IHC	29 28	7 7	7/19 0/9–10
		Zur Hausen et al. (2013)[24]	IHC	21	7	7/18 0/3
Iglambda	B lymphocytes	Murakami et al.(2014)[22]	ISH (mRNA) IHC	29 29	1 0	0–1/19 0/10
		Zur Hausen et al. (2013)[24]	IHC	21	12	12/18 0/3
IgH-R	B lymphocytes	Murakami et al. (2014)[22]	PCR	30	20	10/201 2/10
		Zur Hausen et al. (2013)[24]	PCR	16	16	14/14[b] 2/2
Igkappa	—	Zur Hausen et al. (2013)[24]	PCR	17	17	15/15[c] 2/2[c]
CD117	HSC, MPP, CMP	Sur et al. (2007)[18]	IHC	15	8	NA
CD99	All leukocytes	Sur et al. (2007)[18]	IHC	15	2	NA
		Bhatia et al. (2010)[20]	IHC	23	0	—
CD10	CLP pre-B lymphocytes	Sur et al. (2007)[18]	IHC	15	1	NA
LCA/CD45	Hematopoietic cells (exc. erythrocytes)	Sur et al. (2007)[18]	IHC	15	0	NA
CD20	All B lymphocytes from pro-B cell phase	Sur et al. (2007)[18]	IHC	15	0	NA
CD34	B lymphocyte precursors, myeloid blasts	Sur et al. (2007)[18]	IHC	15	0	NA
CD3	T lymphocytes from pro-thymocytes	Sur et al. (2007)[18]	IHC	15	0	NA
CD44	HSC, leukemic blasts, others	Bhatia et al. (2010)[20]	IHC	19	11	11/17 3/6
CD56	Natural killer cells, activated T cells, neural cells	Bhatia et al. (2010)[20]	IHC	23	19	14/17 5/6

CMP, common myeloid progenitor; IHC, immunohistochemistry; HSC, hematopoietic stem cell; MPP, Multipotent progenitor cells; PCR, polymerase chain reaction; MCV Merkel cell polyomavirus; NA, not applicable.
[a] Monoclonal rearrangement detected in 1 MCV-positive tumor, others were polyclonal.
[b] Monoclonal rearrangement detected in 1 MCV-positive tumor, others were polyclonal.
[c] Monoclonal rearrangement detected in 3 MCV-positive tumors, others were polyclonal.

of origin of MCC might in fact not be the post-mitotic MC, but rather either a pro/pre-B cell or a pre-B cell. Thus, the stage of early B-cell development in which a MCV infection occurs could determine the phenotype and B-cell expression profile of subsequent MCC.[31]

We used immunohistochemistry to evaluate the frequency of PAX5 and TdT expression in a large pool of MCC samples. We also sought to determine whether there is an association between the immunoexpression of PAX5 or TdT (or both) and the presence of MCV DNA and patient and tumor characteristics. The present study validates in a larger cohort the previous smaller scale studies on the connection between MCC and B-lymphoid lineage markers.

2 | PATIENTS AND METHODS

The study protocol was approved by the Ethics Committee of Helsinki University Central Hospital. The Ministry of Health and Social Affairs granted permission to collect patient data and the National Authority for Medicolegal Affairs to collect tissue samples.

2.1 | Patients, clinical data, and tissue samples

Data on patients diagnosed with MCC in Finland from 1979 to 2004 was obtained from the Finnish Cancer Registry and Helsinki University Hospital files. Clinical details were extracted from hospital records. Formalin-fixed, paraffin-embedded tissue blocks were retrieved from the pathology archives. MCC diagnoses were confirmed in a blinded fashion from our earlier studies according to well-established criteria[35] by two researchers with special expertise in MCC pathology.

MCV detection from paraffinized tumor blocks was performed in our previous study and is described in detail elsewhere.[36] In short, the presence of MCV DNA was analyzed from DNA extracted from representative deparaffinized tumor sections. Quantitation of MCV DNA was performed using real-time polymerase chain reaction (PCR). The relative DNA sequence copy number for each tissue sample was expressed as a ratio of MCV DNA to protein tyrosine phosphatase gamma receptor gene DNA. The sample was considered positive whenever MCV DNA copy number per reference gene was greater than 0.1.[37] Tissue microarray (TMA) blocks with 342 tissue cores were used for immunohistochemistry.

2.2 | Immunohistochemistry

Five-micrometers sections were cut from TMA blocks to create three slides from each TMA block; one was stained with hematoxylin and eosin to create a reference slide (Figure 1) and one each was used for immunohistochemical staining of PAX5 and TdT.

Staining for both TdT and PAX5 was performed in a BenchMark XT (Roche Ventana, Tucson, Arizona). For TdT, the antibody used was NCL-L-TdT-339 (clone SEN28) (Novocastra Laboratories Ltd., Newcastle, UK) at 1:50 dilution with an incubation time of 32 minutes at room temperature. For PAX5, the antibody was 790 to 4420 (clone SP34) "Ready to use antibody" (Roche Ventana, Tucson, Arizona); the incubation time was 44 minutes at room temperature. The detection kits were OptiView DAB (Roche Ventana, Tucson, Arizona) and UltraView DAB with amplification kit (Roche Ventana, Tucson, Arizona). In both cases, counterstaining was performed with Mayer's hematoxylin (Lillie's modification) (Agilent, Santa Clara, California) (code S3309) and the slides were cover-slipped with Sakura© Tissue-Tek Film.

2.3 | Immunostaining evaluation

TMA spots were examined and evaluated for expression of PAX5 and TdT by light microscopy. A grading system was used for the expression of PAX5 and TdT in which the spots were classified as showing no expression, weak positive expression, or positive expression. The degree of immunohistochemical expression was determined by the highest degree of expression observed. The slides were evaluated separately by three researchers (BS, HS, and TB) in a blinded fashion.

2.4 | Morphological appearance

TMAs were evaluated by their cytomorphology to three categories; classic, small cell variant and large cell/pleomorphic variant.

2.5 | Statistical analysis

Statistical analysis was performed with NCSS 2007 (NCSS Statistical Software, Kaysville, Utah) software and SPSS statistics 19.0 software (IBM Corporation, New York, New York). *P*-values less than 0.05 were considered significant. Immunoexpression of TdT and PAX5 was compared with gender, age, tumor location, and sun exposure pattern, tumor laterality, disease progression, and hematological malignancy by χ^2 test. The statistical association between the presence of MCV DNA and quantitative PCR results and immunoexpression of TdT and PAX5 were also evaluated statistically against clinical variables, virus copy number, virus positivity by qPCR, and CM2B4 immunohistochemical expression of MCV by Mann-Whitney analysis and Kruskal-Wallis analysis. The cytomorphological features were analyzed statistically against TdT, PAX5 and MCV status by χ^2 test and Kruskal-Wallis one-way ANOVA.

3 | RESULTS

3.1 | Overview of the patients

Detailed patient clinical data are shown in Table 2. Our cohort included 117 MCC patients, of which mean age was 78 years at the time of MCC diagnosis. Eighty-five (72%) of the patients were female. Seven (6%) patients had been previously diagnosed with a hematologic B-cell malignancy and two (1.7%) were kidney transplant recipients. The most common location for the primary tumors was the head and neck region 65 (55%). Chronic non-shield site tumors represented 66 (56%) of all tumors. Half of the tumors (n = 58) were located on the left side of the body. MCV tumor status was available for 113 patients, with 74% of them being MCV-positive. None of the MCC tumors of this study were composite or collision tumors.

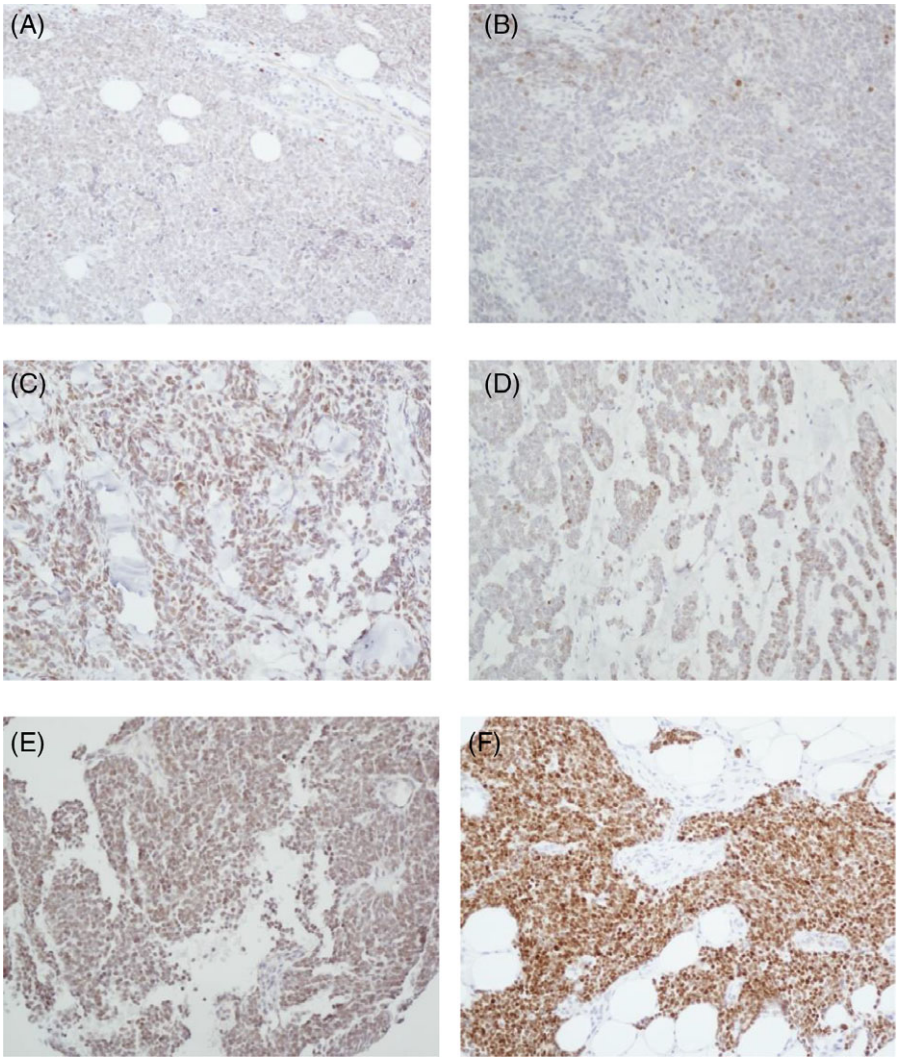

FIGURE 1 Illustration of staining intensities in PAX5 and TdT immunohistochemistry. A, Weak positive PAX5, B, weak positive TdT, C, positive PAX5, D, positive TdT. E and F are examples of some of the strongest staining intensities of PAX5 and TdT, respectively. Original magnification 200×

3.2 | TdT and PAX5 expression in MCC

Table 3 clarifies the expression patterns of TdT and PAX5 stratified by MCV status. In Figure 1 demonstrations of expression patterns are shown. For TdT, we recorded positive immunostaining in 37 (31.6%) samples. We observed strong positivity in 14 samples and weak positivity in 23 samples. Of the 37 positive TdT samples, 34 (91.8%) were also MCV positive. We observed a statistically significant association between virus copy number and positive TdT (P = 0.0056) in Mann-Whitney analysis. In addition, Fisher's exact test revealed a statistically significant association between TdT immunoexpression and MCV virus positivity (P = 0.000495).

Altogether, 26 (22.2%) samples showed positive immunohistochemistry for PAX5. Immunoexpression was strong in six samples and weak in 20 samples. Of the 26 PAX5-positive samples, 19 (73%) were also MCV positive.

Simultaneous immunostaining for TdT and PAX5 was observed in 13 (11.1%) samples. We observed the following three different staining patterns: weak TdT and weak PAX5 in eight samples, strong TdT and strong PAX5 in four samples, and strong TdT and weak PAX5 in one sample. Absent immunostaining for both TdT and PAX5 was observed in 66 (56%) samples.

Twelve out of the 13 (92%) simultaneous TdT and PAX5 expression samples were also MCV positive, although no statistically significant associations were observed because of the small number of samples.

We did not observe any statistically significant associations between TdT or PAX5 immunoexpression and demographics or disease progression—gender, age, tumor location, and sun exposure pattern, tumor laterality, disease progression, and hematologic malignancy.

There were 97 83% of the classic variant, 15 (13%) small cell variant and five (4%) large cell/pleomorphic variant cytomorphology in the TMAs. In the statistical analysis, there were no correlation between cytomorphology features and immunoexpression of TdT and PAX5 or MCV.

4 | DISCUSSION

We studied the immunoexpression of two lymphoid lineage markers (TdT and PAX5) from a large collection, 117 samples, of MCC tumor material. In this study, we attempted to validate in a larger cohort the

TABLE 2 Clinicopathological features of patients

N	117 (%)
Sex	
Male	32 (27)
Female	85 (73)
Age	Range 27-100
≤50 years	3 (2.5)
51-69 years	19 (17)
70-84	59 (50)
85-100	36 (22)
Imunocompromised state (hematologic malignancy)	
NHL	2 (1.7)
CLL	5 (4.3)
Organ transplant	
Kidney	2 (1.7)
Tumor location	
Head and neck	65 (56)
Torso anterior	5 (4)
Torso posterior	10 (8)
Upper extremities	22 (18)
Lower extremities	15 (12)
Tumor laterality	
Left	58 (50)
Right	52 (45)
Midline	1 (0.9)
Not available	6 (5.1)
Disease progression	
Metastasis	41 (35)
MCV status (N = 113)	
Negative	29 (26)
Positive	84 (74)
Not available	4 (3,4)

CLL, Chronic Lymphocytic Leukemia; MCV, Merkel cell polyomavirus; NHL, Non-Hodgkin lymphoma.

previous results of the connections between B-lymphoid markers and MCC. We observed that 31.6% of the 117 MCC samples were positive for TdT (clone SEN28) immunohistochemistry and 22.2% were positive for PAX5 (clone SP34) immunohistochemistry. The results of the present study are quite different from those previously reported.[12,31]

Regarding PAX5, in the previous studies, overall PAX5 expression is detected in 89.5% of 143 MCC samples.[34] All the samples tested by zur Hausen et al. and Murakami et al. in two separate studies were PAX5 positive[31,32] and in the low end of expression, Mhawech-Fauceglia et al. reported 70% expression of PAX5 in 24/34 MCC samples.[27] Thus, our PAX5 positivity is judged low. To derive the reason for the disagreement, we looked at the antibody clones used in those studies. Zur Hausen and Murakami used the same antibody clone—Dako; clone: DAK-Pax5—with parallel 100% PAX5 expression rates,[31,32] further Dong et al. and Mhawech-Fauceglia et al. studies employed the same antibody clone — clone 24, BD Biosciences[16,27] with 93% and 70% positivity, respectively. Hence, at least part of the expression is dependent on the primary antibody used. Obviously, we acknowledge the limitation of using TMA cores in this study, which may have influenced the results. On the other hand, PAX5 expression is typically uniform, with only very few cases showing focal expression.[16] Considering the TdT expression, previous meta-analysis established 65%, 122/187, expression in MCC,[34] ranging from 21%[38] to 76%.[31] Our current expression rate of 32%, falls nicely in this range.

Simultaneous expression of both markers in the present study was scarce and observed only in 11.1% of the samples. Previously, all of the 21 MCC samples that zur Hausen et al. analyzed, showed co-expression of PAX5 and TdT in 72.8% of the samples. These seemingly contradictory findings suggest the possibility that the high rates of PAX5 and TdT expression might be partly explained by chance, given the relatively low number of MCCs studied or antibody clone used. Of note, we observed that PAX5 and TdT expression evaluation in MCC was occasionally confounded by the strong expression of these proteins by tumor-infiltrating leukocytes. Expression of TdT, PAX5, or their simultaneous expression did not correlate statistically significantly with patient or disease parameters — gender, age, tumor location, and sun exposure pattern, tumor laterality, disease progression, or hematologic malignancy.

Corroborating the zur Hausen theory, we observed a strong statistically significant association between virus copy number and TdT expression ($P = 0.0056$) and between MCV positivity and TdT expression ($P = 0.000495$). Previously, Bhatia et al. found a similar relationship between TdT immunohistochemical staining and MCV viral abundance ($P = 0.003$).[38] In about 80% of MCC tumors,[2] oncogenesis is initiated by MCV infection in the cell of origin.[1] For MCV to initiate MCC tumorigenesis a series of specific mutations are required[39]; without these crucial mutations MCV is just a passenger virus. In the present study, the threshold of viral copy numbers determining MCV positivity was set at 0.1, which correlated well with LTA expression with the mouse monoclonal antibody CM2B4. This protocol is generally accepted and widely used.[2,38,40] In our 21 samples that were classified as MCV negative because of threshold, only one was weakly positive for TdT and three for PAX5, thus, further corroborating the finding of the relationship between TdT and MCV.

TABLE 3 Representation of TdT and PAX5 expression patterns stratified by Merkel cell polyomavirus status by PCR, Merkel cell polyomavirus status was available for 113 (96.6%) of the 117 samples

	Expression pattern							
	TdT		PAX5		Simultaneous TdT and PAX5			
MCV status	Negative, n = 80	Positive, n = 37	Negative, n = 91	Positive, n = 26	Both positive, n = 13	Strong TdT and strong PAX5, n = 4	Weak TdT and weak PAX5, n = 8	Strong TdT and weak PAX5, n = 1
MCV+	50	34	65	19	12	4	8	1
MCV−	27	2	25	4	1	NA	NA	NA

Abbreviations: MCV, Merkel cell polyomavirus; NA, Not applicable.

MCC was first described in 1972 by Dr. Cyril Toker as "trabecular carcinoma"[41] and was soon recognized as a potential mimicker of malignant lymphoma.[42] The resemblance of these two malignancies is related to clinical and microscopic appearances.[42,43] The immunohistochemical profile of MCC tumors is somewhat puzzling. The histology of MCC is typical of small round blue cell tumors, an entity that includes a wide variety of the following highly malignant tumors: the Ewing family of tumors, olfactory neuroblastoma, rhabdomyosarcoma, neuroblastoma, lymphoma, desmoplastic small cell tumor, osteosarcoma, small cell lung carcinoma, small cell melanoma, and mesenchymal chondrosarcoma.[44,45] Apart from lymphoblastic neoplasms, TdT is expressed in other small round blue cell tumors.[46] In particular, another neuroendocrine carcinoma, small cell lung carcinoma, frequently shows high TdT expression.[12] Expression of PAX5 in cells other than B lymphocytes has rarely been reported. In small round blue cell tumors, PAX5 is mainly seen in MCC and small cell lung carcinoma.[16,32] Expression of PAX5 has also been observed in certain types of poorly differentiated neuroendocrine carcinomas of the gastrointestinal and pancreatobiliary tracts.[47] However, the expression of PAX5 in MCC has been considered merely a reflection of cancer-associated dysregulation of protein expression than as an indication of lymphocytic origin of MCC.[32]

The immunophenotypic features of MCC are typical of neuroendocrine carcinomas in other sites. Thus, MCC tumors express low-molecular-weight cytokeratins.[48,49] Cytokeratin-20 is the most important and is employed in differential diagnostics, appearing in a dot-like pattern.[50,51] Because of its neuroendocrine differentiation, MCC always stains positively for neuron-specific enolase, a general marker of neuroendocrine tumors.[52,53] Other frequently expressed neuroendocrine markers include chromogranin-A,[54–56] synaptophysin,[56,57] and microtubule-associated protein.[56,58] With regard to lymphoproliferative markers, CD45 is consistently negative in MCC tumors, along with vimentin, CD5, and CD20.[59,60] Three additional lymphoproliferative markers are nonetheless expressed in a significant proportion of MCCs, namely PAX5, CD99, and TdT.[42] However, recent research has revealed that the immunohistochemical features of MCC are to some extent different in MCV-positive and MCV-negative tumors.[61] Immunoglobulin expression seems to be restricted to MCV-positive tumors[31,32] and has been postulated to be induced by MCV infection.[32]

To conclude, we observed frequent TdT and PAX5 immunoexpression in MCC tumor samples. However, concomitant immunoexpression of these markers was scarce. TdT expression was statistically significantly associated with MCV positivity. The absence of a statistically significant association between tumor parameters and disease progression markers undermines the systemic use of these markers in clinical practice.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

ORCID

Virve Koljonen https://orcid.org/0000-0003-0398-4829

REFERENCES

1. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science*. 2008;319(5866):1096-1100.
2. Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. *J Natl Cancer Inst*. 2009;101(13):938-945.
3. Iwasaki T, Matsushita M, Kuwamoto S, et al. Usefulness of significant morphologic characteristics in distinguishing between Merkel cell polyomavirus-positive and Merkel cell polyomavirus-negative Merkel cell carcinomas. *Hum Pathol*. 2013;44(9):1912-1917.
4. Kuwamoto S, Higaki H, Kanai K, et al. Association of Merkel cell polyomavirus infection with morphologic differences in Merkel cell carcinoma. *Hum Pathol*. 2011;42:632-640.
5. Veija T, Sahi H, Koljonen V, Bohling T, Knuutila S, Mosakhani N. miRNA-34a underexpressed in Merkel cell polyomavirus-negative Merkel cell carcinoma. *Virchows Arch*. 2015;466(3):289-295.
6. Veija T, Sarhadi VK, Koljonen V, Bohling T, Knuutila S. Hotspot mutations in polyomavirus positive and negative Merkel cell carcinomas. *Cancer Genet*. 2016;209(1–2):30-35.
7. Motea EA, Berdis AJ. Terminal deoxynucleotidyl transferase: the story of a misguided DNA polymerase. *Biochim Biophys Acta*. 2010;1804(5):1151-1166.
8. Kung PC, Long JC, McCaffrey RP, Ratliff RL, Harrison TA, Baltimore D. Terminal deoxynucleotidyl transferase in the diagnosis of leukemia and malignant lymphoma. *Am J Med*. 1978;64(5):788-794.
9. Orazi A, Cattoretti G, John K, Neiman RS. Terminal deoxynucleotidyl transferase staining of malignant lymphomas in paraffin sections. *Mod Pathol*. 1994;7(5):582-586.
10. Kavalar R, Pohar Marinsek Z, Jereb B, Cagran B, Golouh R. Prognostic value of immunohistochemistry in the Ewing's sarcoma family of tumors. *Med Sci Monit*. 2009;15(8):CR442-CR452.
11. Mathewson RC, Kjeldsberg CR, Perkins SL. Detection of terminal deoxynucleotidyl transferase (TdT) in nonhematopoietic small round cell tumors of children. *Pediatr Pathol Lab Med*. 1997;17(6):835-844.
12. Kolhe R, Reid MD, Lee JR, Cohen C, Ramalingam P. Immunohistochemical expression of PAX5 and TdT by Merkel cell carcinoma and pulmonary small cell carcinoma: a potential diagnostic pitfall but useful discriminatory marker. *Int J Clin Exp Pathol*. 2013;6(2):142-147.
13. Tzorakoleftheraki SE, Iliadis A, Kostopoulos I, Koletsa T. TdT expression in normal and neoplastic sebaceous cells. *Histopathology*. 2017;71:985-988.
14. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of B cell identity and function. *Nat Immunol*. 2007;8(5):463-470.
15. Czapiewski P, Majewska H, Kutzner H, Kazakov D, Renkielska A, Biernat W. TTF-1 and PAX5 are frequently expressed in combined merkel cell carcinoma. *Am J Dermatopathol*. 2016;38(7):513-516.
16. Dong HY, Liu W, Cohen P, Mahle CE, Zhang W. B-cell specific activation protein encoded by the PAX-5 gene is commonly expressed in merkel cell carcinoma and small cell carcinomas. *Am J Surg Pathol*. 2005;29(5):687-692.
17. Bernd HW, Krokowski M, Feller AC, Bartsch S, Thorns C. Expression of terminal desoxynucleotidyl transferase in Merkel cell carcinomas. *Histopathology*. 2007;50(5):676-678.
18. Sur M, AlArdati H, Ross C, Alowami S. TdT expression in Merkel cell carcinoma: potential diagnostic pitfall with blastic hematological malignancies and expanded immunohistochemical analysis. *Mod Pathol*. 2007;20(11):1113-1120.
19. Buresh CJ, Oliai BR, Miller RT. Reactivity with TdT in Merkel cell carcinoma: a potential diagnostic pitfall. *Am J Clin Pathol*. 2008;129(6):894-898.
20. Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW. Merkel cell carcinoma subgroups by Merkel cell polyomavirus DNA relative abundance and oncogene expression. *Int J Cancer*. 2010;126(9):2240-2246.
21. Sidiropoulos M, Hanna W, Raphael SJ, Ghorab Z. Expression of TdT in Merkel cell carcinoma and small cell lung carcinoma. *Am J Clin Pathol*. 2011;135(6):831-838.
22. Murakami I, Takata K, Matsushita M, et al. Immunoglobulin expressions are only associated with MCPyV-positive Merkel cell carcinomas but not with MCPyV-negative ones: comparison of prognosis. *Am J Surg Pathol*. 2014;38(12):1627-1635.

23. Kolhe R, Reid MD, Lee JR, Cohen C, Ramalingam P. Immunohistochemical expression of PAX5 and TdT by Merkel cell carcinoma and pulmonary small cell carcinoma: a potential diagnostic pitfall but useful discriminatory marker. *Int J Clin Exp Pathol*. 2013;6(2):142-147.
24. Zur Hausen A, Rennspiess D, Winnepenninckx V, Speel EJ, Kurz AK. Early B-cell differentiation in Merkel cell carcinomas: clues to cellular ancestry. *Cancer Res*. 2013;73(16):4982-4987.
25. Dong HY, Liu W, Cohen P, Mahle CE, Zhang W. B-cell specific activation protein encoded by the PAX-5 gene is commonly expressed in merkel cell carcinoma and small cell carcinomas. *Am J Surg Pathol*. 2005;29(5):687-692.
26. Mhawech-Fauceglia P, Saxena R, Zhang S, et al. Pax-5 immunoexpression in various types of benign and malignant tumours: a high-throughput tissue microarray analysis. *J Clin Pathol*. 2007;60(6):709-714.
27. Mhawech-Fauceglia P, Saxena R, Zhang S, et al. Pax-5 immunoexpression in various types of benign and malignant tumours: a high-throughput tissue microarray analysis. *J Clin Pathol*. 2007;60(6):709-714.
28. Sur M, Alardati H, Ross C, Alowami S. TdT expression in Merkel cell carcinoma: potential diagnostic pitfall with blastic hematological malignancies and expanded immunohistochemical analysis. *Mod Pathol*. 2007;20(11):1113-1120.
29. Buresh CJ, Oliai BR, Miller RT. Reactivity with TdT in Merkel cell carcinoma: a potential diagnostic pitfall. *Am J Clin Pathol*. 2008;129(6):894-898.
30. Sidiropoulos M, Hanna W, Raphael SJ, Ghorab Z. Expression of TdT in Merkel cell carcinoma and small cell lung carcinoma. *Am J Clin Pathol*. 2011;135(6):831-838.
31. Zur Hausen A, Rennspiess D, Winnepenninckx V, Speel EJ, Kurz AK. Early B-cell differentiation in Merkel cell carcinomas: clues to cellular ancestry. *Cancer Res*. 2013;73(16):4982-4987.
32. Murakami I, Takata K, Matsushita M, et al. Immunoglobulin expressions are only associated with MCPyV-positive Merkel cell carcinomas but not with MCPyV-negative ones: comparison of prognosis. *Am J Surg Pathol*. 2014;38(12):1627-1635.
33. Pan Z, Chen YY, Wu X, et al. Merkel cell carcinoma of lymph node with unknown primary has a significantly lower association with Merkel cell polyomavirus than its cutaneous counterpart. *Mod Pathol*. 2014;27(9):1182-1192.
34. Sauer CM, Haugg AM, Chteinberg E, et al. Reviewing the current evidence supporting early B-cells as the cellular origin of Merkel cell carcinoma. *Crit Rev Oncol Hematol*. 2017;116:99-105.
35. Sahi H, Koljonen V, Kavola H, et al. Bcl-2 expression indicates better prognosis of Merkel cell carcinoma regardless of the presence of Merkel cell polyomavirus. *Virchows Arch*. 2012;461(5):553-559.
36. Schrama D, Peitsch WK, Zapatka M, et al. Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma. *J Invest Dermatol*. 2011;131(8):1631-1638.
37. Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H. Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. *Clin Cancer Res*. 2011;17(14):4806-4813.
38. Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW. Merkel cell carcinoma subgroups by Merkel cell polyomavirus DNA relative abundance and oncogene expression. *Int J Cancer*. 2010;126(9):2240-2246.
39. Shuda M, Feng H, Kwun HJ, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci U S A*. 2008;105(42):16272-16277.
40. Higaki-Mori H, Kuwamoto S, Iwasaki T, et al. Association of Merkel cell polyomavirus infection with clinicopathological differences in Merkel cell carcinoma. *Hum Pathol*. 2012;43(12):2282-2291.
41. Toker C. Trabecular carcinoma of the skin. *Arch Dermatol*. 1972;105(1):107-110.
42. Wick MR, Santa Cruz DJ, Gru AA. Non-lymphoid lesions that may mimic cutaneous hematopoietic neoplasms histologically. *Semin Diagn Pathol*. 2017;34(1):99-107.
43. Sibley RK, Rosai J, Foucar E, Dehner LP, Bosl G. Neuroendocrine (Merkel cell) carcinoma of the skin. A histologic and ultrastructural study of two cases. *Am J Surg Pathol*. 1980;4(3):211-222.
44. Tarkkanen M, Knuutila S. The diagnostic use of cytogenetic and molecular genetic techniques in the assessment of small round cell tumours. *Current Diagnostic Pathology*. 2002;8(5):338-348.
45. Pisick E, Skarin AT, Salgia R. Recent advances in the molecular biology, diagnosis and novel therapies for various small blue cell tumors. *Anticancer Res*. 2003;23(4):3379-3396.
46. Magro G, Longo FR, Angelico G, Spadola S, Amore FF, Salvatorelli L. Immunohistochemistry as potential diagnostic pitfall in the most common solid tumors of children and adolescents. *Acta Histochem*. 2015;117(4–5):397-414.
47. Ainechi S, Mann SA, Lin J, et al. Paired Box 5 (PAX5) Expression in poorly differentiated neuroendocrine carcinoma of the gastrointestinal and pancreatobiliary tract. *Appl Immunohistochem Mol Morphol*. *Appl Immunohistochem Mol Morphol*. 2018;26(8):545-551.
48. Moll R, Osborn M, Hartschuh W, Moll I, Mahrle G, Weber K. Variability of expression and arrangement of cytokeratin and neurofilaments in cutaneous neuroendocrine carcinomas (Merkel cell tumors): immunocytochemical and biochemical analysis of twelve cases. *Ultrastruct Pathol*. 1986;10(6):473-495.
49. Miettinen M, Lehto VP, Virtanen I, Asko-Seljavaara S, Pitkanen J, Dahl D. Neuroendocrine carcinoma of the skin (Merkel cell carcinoma): ultrastructural and immunohistochemical demonstration of neurofilaments. *Ultrastruct Pathol*. 1983;4(2–3):219-225.
50. Su LD, Lowe L, Bradford CR, Yahanda AI, Johnson TM, Sondak VK. Immunostaining for cytokeratin 20 improves detection of micrometastatic Merkel cell carcinoma in sentinel lymph nodes. *J Am Acad Dermatol*. 2002;46(5):661-666.
51. Moll R, Lowe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol*. 1992;140(2):427-447.
52. Leong AS-Y, Phillips GE, Pieterse AS, Milios J. Criteria for the diagnosis of primary endocrine carcinoma of the skin (Merkel cell carcinoma). A histological, immunohistochemical and ultrastructural study of 13 cases. *Pathology*. 1986;18(4):393-399.
53. Sibley RK, Dahl D. Primary neuroendocrine (Merkel cell?) carcinoma of the skin. II. An immunocytochemical study of 21 cases. *Am J Surg Pathol*. 1985;9(2):109-116.
54. Wilson BS, Lloyd RV. Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. *Am J Pathol*. 1984;115(3):458-468.
55. Haneke E, Schulze HJ, Mahrle G. Immunohistochemical and immunoelectron microscopic demonstration of chromogranin A in formalin-fixed tissue of Merkel cell carcinoma. *J Am Acad Dermatol*. 1993;28(2):222-226.
56. Koljonen V, Haglund C, Tukiainen E, Bohling T. Neuroendocrine differentiation in primary Merkel cell carcinoma--possible prognostic significance. *Anticancer Res*. 2005;25(2A):853-858.
57. Buffa R, Rindi G, Sessa F, et al. Synaptophysin immunoreactivity and small clear vesicles in neuroendocrine cells and related tumours. *Mol Cell Probes*. 1987;1(4):367-381.
58. Liu Y, Mangini J, Saad R, et al. Diagnostic value of microtubule-associated protein-2 in Merkel cell carcinoma. *Appl Immunohistochem Mol Morphol*. 2003;11(4):326-329.
59. Wong HH, Wang J. Merkel cell carcinoma. *Arch Pathol Lab Med*. 2010;134(11):1711-1716.
60. Trenkic Bozinovic M, Krasic D, Katic V, et al. Comparative analysis of clinicopathological and immunohistochemical characteristics of Merkel cell carcinoma. *J Buon*. 2014;19(2):530-534.
61. Jankowski M, Kopinski P, Schwartz R, Czajkowski R. Merkel cell carcinoma: is this a true carcinoma? *Exp Dermatol*. 2014;23(11):792-794.

How to cite this article: Johansson B, Sahi H, Koljonen V, Böhling T. The expression of terminal deoxynucleotidyl transferase and paired box gene 5 in Merkel cell carcinomas and its relation to the presence of Merkel cell polyomavirus DNA. *J Cutan Pathol*. 2018;1–7. https://doi.org/10.1111/cup.13372